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Abstract

Monosodium glutamate (MSG) is known to enhance liking for the flavor of savory foods, but whether associations between flavors and effects of MSG lead to changes in subsequent liking and intake for the flavor alone is unclear. To test this, 32 volunteers evaluated and consumed a novel savory soup with no added MSG before and after four training sessions where the same soup was consumed either unchanged (Control) or with added MSG. The addition of MSG during training increased both pleasantness and savory character of the soup and resulted in a larger increase in rated pleasantness of the soup in the MSG-trained relative to control condition when the soup was re-evaluated post-training without MSG. There was also a significant increase in voluntary soup intake post-training after the soup had been paired with MSG but not in the Control condition, and rated hunger increased more after tasting the soup post-training in the MSG-trained but not Control condition. These findings demonstrate that co-experience of a savory flavor and MSG can result in increased subsequent liking and intake for the flavor in the absence of MSG, and possible explanations for how MSG reinforces learning are discussed.

Key terms: Monosodium glutamate Flavor-flavor learning Taste hedonics Flavor liking

Introduction

Of the many explanations of how liking for flavors may be acquired, two learning models have attracted particular attention [see 1, 2 for recent reviews]. According to the flavor-consequence learning (FCL) model, co-experience of a flavor (the conditioned stimulus, CS) and a positive post-ingestive effect of the ingested food or drink (acting as unconditioned stimulus, US) leads to a conditioned increase in flavor liking. In human studies, consequences which have been reported to increase flavor liking in this way include the post-ingestive effects of the major macronutrients carbohydrate, fat and protein, and the effects of caffeine. In contrast, in the flavor-flavor learning (FFL) model of flavor preference acquisition, associations between novel flavor elements (CS) with existing liked or disliked flavor elements (US) result in change in liking for the CS in line with the evaluation of the US. The most frequently used US in studies of FFL have been sweet and bitter tastes, where the innate liking for sweet and dislike of bitter seen in humans and other animals generates a strong affective response [3]. Thus the sweet taste of sucrose can reinforce increased liking for sucrose-paired flavors and odors in humans [4-7] and other animals [8-13], although the ability of sucrose to support increased liking in humans does depend both on the individual affective responses to sucrose [5] and hunger state at the time of testing . Likewise, repeating pairing of food-related CS with the bitter taste of quinine can lead to reduced liking for the CS [5], and the sour taste of citric acid may also lead to reduced liking for flavor-related CS (food odors: [14]).

The only studies to date which examined FFL in humans using the other two major tastants (salt and monosodium glutamate, MSG) did not find evidence for increased flavor liking. In one study, participants repeatedly tasted, but did not consume, small samples of a novel flavor combined with MSG before and after evaluating the MSG-paired flavor, and two control flavors [15]. There was no evidence of any consistent change in liking for the MSG-paired flavor relative to the two control flavors. In contrast, repeated pairing of a savory odor with a salty US based on a combination of NaCl and MSG resulted in reduced liking for the flavor [5], in line with the aversive nature of the NaCl/MSG US. Thus even though the addition of MSG to food enhances liking for the overall food flavor [15-17], and MSG has long been manufactured as a flavor enhancer, to date there is no evidence that MSG can enhance liking through FFL. However, while MSG can enhance liking for congruent savory food flavors [15, 17, 18], MSG presented alone is not perceived as a pleasant experience [18, 19]. The recent finding that the specific combination of a hedonically neutral MSG stimulus and an unpleasant vegetable odor produced a positive hedonic response [18], demonstrates that flavor liking in the context of MSG reflects a synergistic interaction between MSG and congruent flavor components. It may be that the previous failures to find evidence of increased flavor liking following pairing with MSG may thus reflect an insufficiently liked CS-US combination, suggesting that MSG may support increased flavor-liking through FFL but only where the CS-US combination is both congruent and liked.

In contrast to the lack of evidence for enhanced flavor-liking induced by flavor-MSG pairings through FFL, there is evidence that MSG can support increased flavor-liking induced by FCL [15]. This was shown in two separate experiments. In the first, participants consumed both a novel flavored soup with added MSG and a different soup flavor without MSG on seven alternate days. They evaluated liking for both soup flavors before and after pairing with MSG, and in contrast to the lack of change in liking when these soups were tasted only, liking for the MSG-paired flavor increased when the training included consumption of MSG. A second experiment confirmed these findings. Thus repeated experience of a novel savory flavor with MSG resulted in significant increases in flavor liking. The primary aim of the present experiment was to replicate and extend this finding. In the earlier studies, the only measures of change in responses to the flavor CS paired with MSG was based on flavor evaluation. An important question is whether altered flavor liking induced by MSG consumption also results in subsequent stimulation of appetite, and so increased intake. It is well known that artificially increasing flavor pleasantness can increase short-term food intake [see 20 for review], but no study to date has explicitly tested in humans the extent to which conditioned increases in flavor liking also impact on intake. Thus the present study extended the previous investigations of flavor-acceptance learning [15] facilitated by MSG by measuring both changes in flavor evaluation and intake following flavor-MSG pairings. In addition, since it is known that hunger state can modify expression of acquired flavor liking [6], we also controlled more carefully the hunger state of participants throughout the study to reduce the possibility that apparent effects of MSG were not confounded by spurious differences in hunger state.

As well as altering liking, repeated pairings of flavor or food-odor CS with tastant US in humans can result in the CS acquiring taste-like properties. Thus, food-related odor CS which have been paired with a sweet taste US are rated as smelling sweeter in subsequent tests [5, 6, 14, 21-23] with these changes enduring even after multiple exposures to the CS alone post-training [23]. These effects are not restricted to sweetness: food-related odors are rated as smelling more sour after pairing with citric acid [14, 21], and more bitter after pairing with quinine [5]. More recently, these changes in the quality of food related odors have been extended to the rated quality of the CS flavor in the mouth, with significant increases in the bitterness of flavor CS paired with quinine [24], and trends for increased sweetness for flavors paired with sucrose or aspartame [4, 24]. However, pairing food related odors with a combination of MSG and NaCl did not result in significant increases in the rated saltiness or savoriness of the odor CS [5]. Whether this represents a qualitative difference between NaCl and MSG and other tastants, or reflects a difficulty in attribution of salt-like qualities to odors, is unclear. In the only studies to date to report enhanced flavor-liking based on MSG-flavor pairings [15], no measures of flavor quality were taken. Given our recent success in identifying changes in the experienced quality of food flavors in the mouth after pairing of these flavor CS with bitter and sweet tastes [4, 24], the further aim of the present study was to explore whether a novel savory flavor CS acquired MSG-like flavor properties following repeated pairings of the flavor CS and a MSG US.

Method

Design

The study used a between-subjects design to contrast changes in voluntary consumption, hedonic and sensory evaluations of the flavor of a target savory soup on two test days before and after four training days where the soup was either consumed either unaltered (exposure alone Control condition) or with its flavor enhanced by the addition of 0.5% of MSG (MSG experimental condition).

Participants

Thirty two volunteers (27 women and 5 men) were recruited from staff and students at the University of Sussex. Potential participants had previously completed a general recruitment questionnaire which included questions from the Three Factor Eating Questionnaire [TFEQ: 25] and details of all food allergies and aversions. Since some studies have suggested that highly restrained individuals may be insensitive to flavor-based learning [26, 27], those scoring more than six on the TFEQ restraint scale were excluded. Potential participants were sent an information sheet incorporating a list of food ingredients and exclusion criteria, and those with allergies or aversions to any of the listed food ingredients, who suffered from diabetes or who had a diagnosed eating disorder were excluded. Participants were allocated at random to one of two training conditions based on the addition of 0.5% MSG or nothing (control) to the soup during training. The two groups had similar gender ratios (MSG consisted of 13 women and 3 men, Control 14 women and 2 men), and did not differ significantly in age (MSG: 22.3 ± 2.2 years, Control: 24.9 ± 2.3, $t(30) = 0.83$, NS) or BMI (MSG: 21.4 ± 0.3, Control: 22.1 ± 0.5, $t(30) = 1.17$, NS).

Test food

The soup used for the test sessions was a proprietary brand low-energy soup ('Organic Soup in a Mug Leek and Potato flavour', Just Wholefoods, UK). This was selected from extensive pilot studies, which identified the soup as having a savory character, and to be both neither too unpleasant nor pleasant (between 40 and 65pt on 100pt line scales anchored with "Very unpleasant" and "Very pleasant") and relatively novel in flavor (>55pt on 100pt novelty rating). Each sachet of soup flavorings were combined with 200g of boiling water. The served soup had an energy density of 21.1 kcal/100g, mainly derived from carbohydrate (nutrient content per 100g: carbohydrate 4.2g, fat 0.2g, protein 0.6g, sodium 0.3g).

The test soup was consumed *ad libitum* during the pre- and post-training sessions, but during training sessions a fixed serving of 200g was consumed either unaltered (Control condition) or with the addition of 0.5% w/w MSG (MSG condition). This concentration of MSG was selected since it was effective in generating changes in liking in a previous study of flavor-acceptance learning [15]. All soup was served in white ceramic soup bowls at a temperature of 60-65°C.

Sensory and hedonic evaluations and intake measurements

The main data were a combination of sensory and hedonic evaluations, and measured intake, of the test CS soup before and after training. All measurements were made in individual testing cubicles equipped with Sussex Ingestion Pattern Monitors (SIPM), which consists of a concealed digital balance (Sartorius BP 4100) connected via a serial line to an Apple G3 computer, custom-programmed using Future Basic II (Staz Software) to read the balance weight on stability to an accuracy of 0.1 g, at two-second intervals during feeding bouts. Weight data and subjective ratings (see below) were passed continuously to a data file. This combination of hardware and software for this system was developed at Sussex, based on a modification of the Universal Eating Monitor developed by Kissileff [28] and has been used extensively to measure human ingestive behavior [29-31] including studies using soup [32-34].

Using the SIPM software, participants were first required to record a set of mood and appetite ratings, using digital Visual Analogue Scales (VAS) presented on the SIPM. VAS ratings comprised of a series of questions in the form, "How <word> do you feel?" with the end-anchors "Not at All" (recorded as 0) and "Extremely" (recorded as 500). Ratings were completed by moving a cursor to the appropriate point on the horizontal VAS and registering their selection using the computer mouse. Participants were required to rate how calm, clear-headed, drowsy, energetic, full, headachy, hungry, lively, nauseous, thirsty, and tired they felt. Participants were then presented with a bowl of the test soup (c. 220g) and were required to eat a single spoonful. They then completed a second set of VAS evaluations in the form "Rate the following property of this food" with the word describing the hedonic and sensory qualities of the soup to be rated (bitter, novel, pleasant, salty, sour, savory and sweet) presented below the question. Once these ratings were completed, participants were instructed to eat *ad libitum* ("Please eat as much as you like"), and they signaled the end of the meal by clicking a button ("done") on the computer screen, which triggered a second set of mood VAS ratings.

Procedure

Participants attended the Ingestive Behaviour Unit at Sussex University on seven non-consecutive days. Day one was a practice session (participants were not informed of this) in which participants consumed a different soup to that used in test sessions (Tomato miso soup, Free & Easy, UK). The purpose of this session was to allow participants to become familiar with the software, and to prevent exclusion of participants due to error. Days two and seven were test days (the Pre- and Post-training sessions), and days 3-6 were training sessions. On all seven days, participants were required to eat nothing and drink only water from 2300h on the previous evening, and to report to the Unit at a pre-assigned time between 0830 and 1000h for breakfast. Breakfast consisted of 60g cereal (Crunchy Nut Cornflakes, Kelloggs brand) along with 160g of semi-skimmed milk and 200g of orange juice (total 1682 kJ). Once breakfast was complete, participants were free to leave the Unit and were required to return three hours later, having consumed only water, for the main test session.

Pre- and post-training (Days 2 and 7)

On their return to the Unit on the test days, participants were taken to a SIPM test cubicle, and were instructed to follow the on-screen instructions which directed them to complete the initial set of appetite and mood ratings before calling their experimenter. They were then served a bowl of the test soup, which they evaluated for hedonic and sensory properties and then consumed it *ad libitum*. Participants were interrupted automatically by the SIPM system after consumption of every 50g of soup and asked to rate hunger, fullness and thirst. Additional bowls of soup (served at 60-65°C) were provided after every 150g consumed until the participants had consumed as much as they liked, at which point mood and appetite were re-rated. The use of refills in this way prevented participants simply emptying the bowl and then ending the session, and so allowed a more accurate measure of voluntary intake. Once the ratings were complete, participants were free to leave on the Pre-training day, but completed a brief structured debriefing and had their height and weight measured in light clothing before being paid on the final (Post-training) session.

Training (Days 3-6)

The only difference between the instructions on the training and test sessions was the instructions provided during the soup consumption phase. During the test sessions, soup intake was *ad libitum*, whereas on training sessions participants were served a fixed quantity (200g) of soup, and were instructed to consume all of this. Also, the only ratings collected on training days were those before soup were served, when soup was first tasted and when the meal was complete (i.e. there were no interruptions in eating to make additional ratings).

Statistical analyses

Since the primary focus was on changes in response to the soup at post-training as a consequence of exposure to the soup in the MSG and Control conditions, initial analyses confirmed that there were no spurious group differences in hedonic or sensory evaluation of the soup at Pre-training using between samples t-tests. These data were converted into change scores by subtracting the equivalent Pre-training data, and these change data were then contrasted between training conditions using t-tests. Intake data at Pre and Post-training were contrasted by 2-way ANOVA, with session (Pre- or Post-training: within-subject) and condition (MSG or control as factors: between-subject). Hunger ratings at the start and end of soup intake at Pre and Post-training were contrasted by 3-way ANOVA, with session, and time of rating within-subject and condition between subject. One index of the extent to which the sensory quality of a food impact on appetite is the extent to which the experience of hunger changes on food presentation. To assess the impact of the soup in this way, change in hunger following initial tasting of the soup was calculated at Pre and Post-training by subtracting rated hunger before soup was presented from rated hunger once the soup had been tasted as a test of the appetizing effect of the soup flavor [35]. These changes were then contrasted between the two training conditions at the two test sessions using mixed two-way ANOVA.

Responses during training were analyzed to ensure that the MSG manipulation had the expected effects on flavor quality and liking. Hedonic and sensory evaluations of the soup during training were contrasted between conditions across the four training sessions using 2-way mixed ANOVA. Hunger and fullness ratings at the start and end of soup ingestion were also contrasted between conditions and training sessions using 3-way ANOVA, with condition between-subjects and time (start or end of meal) and session (pre or post) within-subjects.

Results

Responses at Pre and Post-training sessions

Between samples t-tests confirmed that there were no differences in ratings of the sensory or hedonic properties of the soup between the two conditions at the Pre-training session (Table 1). The change in rated pleasantness of the soup between Post- and Pre-training sessions differed significantly between training conditions ($t(30) = 2.85$, $p=0.008$), with liking increasing in the condition which had added MSG during the intervening training sessions, but decreasing in the Control condition (Figure 1a). This change in liking was not accompanied by any equivalent change in evaluation of the savory-qualities of the soup, with no significant differences between conditions in changes in ratings of how savory (Figure 1b) or salty (Figure 1c) the soup was rated. There were also no differences between conditions in evaluation of the soup at Pre- or Post-training in terms of other taste-like properties (sweet, sour and bitter: Table 1).

The amount of soup consumed at Pre- and Post-training sessions depended on an interaction between condition and session [$F(1,30) = 6.02$, p=002]. There was no significant difference in intake between conditions at the pre-training session ([$F(1,30) = 0.02$, NS]: MSG training condition 197.4 ± 40.2g, Control training condition 205.6 ± 41.3g), but the change in intake between pre- and post-training sessions (Figure 1d) differed significantly between conditions ($t(30) = 2.45$, $p<0.05$), with intake increasing after training in the MSG condition but tending to decrease in the Control condition.

Since liking for food flavors can be affected by hunger state at the time of testing [6], any difference in hunger between groups at either Pre- or Post-training could have produced spurious effects on the evaluative and intake measures. However, analysis of hunger at the start and end of the Pre and Post-training sessions (Table 2) revealed no significant difference between conditions [$F(1,30) = 0.36$, NS], or session [$F(1,30) = 1.73$, NS] or any interaction between these effects [$F(1,30) = 0.18$, NS]. There was, as expected, a large effect of time of rating (before or after the meal: $F(1,30) = 25.23$, $p<0.001$], and a marginal interaction between session and time [$F(1,30) = 4.05$, $p=0.053$], with a tendency for hunger to decrease less after eating at the Post- compared with Pre-training sessions in both conditions despite greater intake in the condition which had MSG added during training. The increased liking for the CS as a consequence of training in the MSG condition could have increased the ability of the CS to enhance appetite (the appetizer effect, [35]). To test this, the change in hunger from before soup was presented to when it was first tasted was calculated, and contrasted between conditions at the two training sessions. Analysis of these changes in hunger revealed a significant interaction of condition and time of rating [$F(1,30) = 4.30$, $p=0.047$]. As can be seen (Figure 2), tasting the soup caused similar, small increases in hunger in both conditions during the Pre-training session, but there was a marked increase in appetite after tasting the soup in the condition where MSG was added during training which was significantly greater than that in the Control condition [F(1,29) = 6.64, p=0.015], at the Post-training session.

Responses during the training sessions

For the addition of MSG to the soup during training to be an effective hedonic US, evaluations of the soup during training (Table 3) in the MSG condition should be more positive than should liking by the Control group. Analysis of pleasantness across the four training trials confirmed that this was so: pleasantness ratings differed significantly between conditions across the four training sessions [F(1,30) = 10.02, p= 0.004], with higher ratings in the MSG condition than in the Control condition. There was no effect of training day on these evaluations. Analysis of the rated savory quality of the soup during training also confirmed that the addition of MSG significantly increased the savory quality of the soup ([F(1,30) = 5.87, p=0.022]. The soup with added MSG was not experienced as more salty [F(1,30) = 0.05, NS], sweet [F(1,30) = 1.47, NS], sour [F(1,30) = 0.10, NS] or bitter [F(1,30) = 0.31, NS] than in the Control condition. Thus the addition of MSG appeared to increase pleasantness purely by modifying the savory quality of the soup flavor in the MSG condition.

Analysis of ratings of hunger and fullness at the start and end of the four training sessions (Table 2) revealed no significant main effects or interactions involving condition or time (no p<0.10), but as expected overall hunger was less [F(1,30) = 52.98, p<0.001], and fullness greater [F(1,30) = 50.48 p<0.001], after consuming the soup. Thus there was no evidence of any significant effects of the added MSG on appetite within the training sessions.

Inter-relationship between variables

In order to explore how intake related to perceived sensory and hedonic qualities of the soup, the relationship between these variables was explored using correlational analyses. Given the clear relationship between palatability and intake in other studies [see 20 for recent review], it was predicted firstly that intake would be related to liking overall, and secondly that increased intake post-training for the MSG-paired soup was a consequence of increased liking. Intake was positively correlated with rated soup pleasantness both at Pre-training ($r(32) = 0.48$, $p=0.006$) and at Post-training ($r(32) = 0.50$, $p=0.004$), and this effect was still significant when the training condition was partialled out (Pre-training $r(29) = 0.48$, $p=0.007$: Post-training $r(29) = 0.45$, $p=0.011$). In contrast, none of the sensory evaluations (savory, salt, sour, sweet or bitter) correlated significantly with voluntary soup intake (Table 4). In terms of relationships between sensory variables, only rated savoriness correlated with pleasantness (Pre-test ($r(32) = 0.36$, $p=0.043$): Post-test $r(32) = 0.41$, $p=0.02$). Surprisingly, rated savoriness was unrelated to the other sensory characteristics, while saltiness correlated with ratings of sweet and sour Pre-test, but bitter and sour post-test, while sourness and bitterness were positively correlated throughout (Table 4). As a final test of what changes in perceived sensory character of the soup predicted changes in intake, changes in intake, pleasantness and sensory evaluation of the soup at Post-training were contrasted, with separate analyses conducted on each group (Table 5). None of the changes in evaluations of flavor characteristics were correlated significantly with changes in intake, although rated pleasantness tended to be positively correlated with intake in both groups, and the restricted power in these analyses may have masked these effects. In terms of changes in sensory quality, the only significant correlations were a negative correlation between changes in ratings of savory and bitter, and a positive correlation in changes in ratings of bitter and sour, in the Control group, and a positive correlation between changes in ratings of sour and salty in the MSG-paired group. Again, the sample size limited the scope of these analyses and other relationships may have been significant if a larger sample had been tested.

Discussion

The key finding of the current study was that repeated experience of a low energy soup with the taste of MSG resulted in enhanced pleasantness of the flavor and intake of the same soup when tested without MSG. Thus these data confirm the previous finding that consumption of a novel soup with added MSG can result in increased flavor-liking [15], and show that this increased liking is also associated with increased consumption of the MSG-paired flavor. Thus flavor-based learning facilitated by MSG has effects both on flavor evaluation and consumption.

The similarity in increased liking for the MSG-paired flavor in the present study following repeated consumption of soup with added MSG and that in the previous study [15] suggests that changes in flavor liking induced by MSG are robust. They also contrast with the acquired dislike for orthonasal evaluations of savory odors before and after repeated retronasal pairing of the same odor along with a combined MSG/NaCl US [5], where odor liking decreased significantly. The combined MSG plus soup flavor stimulus used in training in the present study was perceived as a pleasant overall flavor, in contrast to dislike of the combination of food odor with the NaCl/MSG combination [5]. In addition, only MSG was used as taste US in the current study, while training involved consumption of a large quantity of the combined soup+MSG stimulus here, but only involved oral experience of the NaCl/MSG plus odor stimuli in a taste-and-spit procedure in our previous study. Thus the present study provided greater oral exposure to a liked CS/US combination, and this probably explains the contrast between acquired liking in the present study and acquired aversion in the previous study. Moreover, the present study examined evaluations of the actual flavor of the soup in the mouth, whereas the previous study examined pleasantness of a food odor alone. It is possible that the additional sensory cues in the mouth (texture, temperature etc.) in the current study may have both contributed to the overall experience of liking for the CS/US combination, as well as potentially aiding encoding of this association.

As well as changes in liking, the present study found evidence of increased intake of the soup post-training in the MSG but not Control group. This finding can be explained as a simple consequence of the increase in liking, since it is well documented that intake is greater for food with more pleasant flavors [36]. One marker of the effects of palatability on intake is the change in the experience of appetite when food is first experienced [35]. In the present study, rated hunger increased significantly on tasting the food for the group trained with MSG post-training, but not pre-training, providing clear evidence that the acquired liking for the MSG-paired flavor resulted in an enhanced ability of the soup flavor to stimulate appetite. Another notable feature of changes in rated appetite associated with consuming the soup was that in both the MSG-paired and Control conditions, hunger declined less on consumption post-training relative to pre-training, perhaps suggesting that participants had learned by post-training that the food was les satiating than they had expected when first consumed, in line with the predictions of conditioned satiety [2].

It might have been expected that re-exposure to the soup without MSG post-training might have resulted in a negative contrast effect, where the experience of a less pleasant flavor results in decreased intake, similar to that reported, for example, when rats fed on a preferred cafeteria diet are switched back to an unvaried chow diet [37]. The absence of any such effect here, even though the average pleasantness of the soup post-training was lower than the soup with added MSG during training, argues that the acquired changes in flavor evaluation were greater than was any negative effect of short-term sensory contrast. An important question is how enduring these learned changes in evaluation and intake of the soup are, and future studies might explore effects of repeated consumption of the soup alone post-training. In terms of the liking change, studies of evaluative conditioning which appear to share many of the properties of FFL suggest that liking acquired by associations at the sensory level may be resistant to extinction [38], as do the changes in sensory quality of odors paired with tastants [23]. In contrast, the only study to explore the enduring nature of an acquired liking for a flavor by pairing with a consequence (caffeine) found evidence that this acquired liking disappeared when the flavor was consumed repeatedly without any consequence [39]. This implies that the enduring nature of the change in liking seen here will depend crucially whether this was acquired by FFL, FCL or a combination of these processes.

A critical question is what underlies the ability of MSG to support these changes in response to the MSG-paired flavor. In the present study MSG had clear effects on the evaluation of the soup during training: thus participants who consumed the soup with added MSG rated the overall flavor as more pleasant and savory than did the control group who consumed the same flavored soup without added MSG. Thus one possibility is that the subsequent change in evaluation of the flavor alone following pairing with MSG reflects an association at a sensory level between the liked flavor-MSG combination used during training. Thus the change in liking might result from FFL based on the association between the flavor and the positive sensory quality during training. However, in the previous investigation of flavor-based learning with MSG, when the flavor-MSG combination was tasted but not ingested [15], no significant change in liking was reported, suggesting that sensory exposure alone was not sufficient. It could however be that exposure to 10ml samples in a taste-only paradigm in that study did not give sufficient flavor-exposure to the flavor-MSG combination for evidence of FFL to emerge, and inspection of data from that study (Experiment 2) suggests that there was some evidence for increased liking for the flavor paired with MSG in the taste-only condition, but that the magnitude of change was too small to be detected by the power of that study. In the present study, consumption of 200ml on four occasions would have given much greater flavor-exposure, and so increased the chances of detecting a positive FFL effect.

The alternative explanation to FFL for the present findings would be that the changes in liking and intake of the MSG-paired flavor arose as a consequence of a post-ingestive effect of MSG (as a consequence of FCL). The question then is what are the likely post-ingestive effects of MSG. Since the ability of macronutrients to support FCL are based on the ability of the ingested energy to modify appetite [1, 2], one possibility is that MSG acted to reduce subsequent appetite, and it was this appeasement of appetite which reinforced changes in flavor-liking. Human studies suggest this possibility is unlikely, however, since there was no evidence of any differences in appetite after consuming the MSG relative to control soup (Table 2), and a study which looked explicitly for acute effects of MSG on appetite failed to find any effect of MSG on subsequent food intake [40]. A more recent long-term trial, where elderly participants consumed an additional 300mg MSG daily for sixteen weeks also found no effects on energy intake [41]. Thus there is no evidence that MSG has acute or long-term effects on appetite in humans which could explain how MSG might condition flavor-liking through FCL. However, the metabotropic glutamate receptor subtype mGlu5 is known to be involved in modulation of central reward pathways [42], and mice lacking this receptor weighed less, and responded less to acute food deprivation, than did mice with the mGlu5 receptor [43], suggesting a role for glutamate in appetite control in animals. Whether dietary glutamate could stimulate these central receptors is unclear, however, but if so this could offer an explanation for the ability of MSG to support changes in flavor-liking through FCL. Repeated consumption of glutamate does alter plasma concentrations of amino acids, particularly with increases in the amino acids taurine, alanine and ornithine [44], and a further possibility might be that these changes produce effects which are rewarding, perhaps by modulation of neurotransmitter function.

While liking and intake of the MSG-paired soup were both increased post-training, there was no evidence that the soup acquired MSG-like sensory qualities. This contrasts with clear evidence of increased sweetness for flavors and food-related odors following repeated pairing with a sweet taste [5, 6, 14, 21-24], but replicates our earlier finding of no change in evaluation of food-related savory odors after pairing with a combined NaCl/MSG US [5]. Inspection of the data for salty and savory evaluations (Table 1) does suggest a weak trend for increased salty and savory ratings for the soup flavor after pairing with MSG, and similar weak trends were evident in our earlier study [5]. Correlational analyses did not, however, find evidence that changes in savory ratings were related to changes in saltiness, and exploration of the inter-relationships between the different taste qualities assessed here did not reveal any clear pattern within sensory qualities or between changes in sensory evaluation and intake. It is well known that multiple taste stimuli are hard to discriminate in the context of complex flavor stimuli, with suppression of perceived intensity of dissimilar tastes [45]. Notably, the ability to detect the presence of MSG in mixtures containing more than two other taste stimuli was at chance level [46]. Thus the complexity of the current flavor stimuli may have masked subtle changes in perceived sensory quality brought about by the association of the overall flavor of the soup and the taste of MSG in the MSG-trained group. This contrasts with studies of olfactory conditioning, where odor-taste pairings are usually limited to a single tastant (typically sweet: [5, 6, 14, 21-23]), so avoiding these mixture-suppression effects. The finding that an odor which was repeatedly paired with orosensory experience of fat resulted in increased ratings for the creaminess of the fat-associated odor [47] provides evidence for acquisition of a savory-like quality by a flavor component. Future explorations of potential savory qualities of food flavors by association with the taste of MSG may need to use less complex flavor CS.

In summary, the present study provides further evidence that liking for savory flavors can be enhanced by association with MSG, but whether these changes reflect a sensory or post-ingestive effect of MSG requires further substantiation.

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Table 1. Evaluations of the hedonic and sensory qualities of test soup at the Pre- and Post-training sessions in the condition where MSG was added during training and Control. All values are mean ± SEM, n=16.

Attribute evaluated	MSG training condition		Control training condition	
	Pre-training	Post-training	Pre-training	Post-training
Pleasantness	324 ± 29	371 ± 23	332 ± 26	300 ± 28
Savory	380 ± 34	395 ± 36	377 ± 25	365 ± 22
Salty	258 ± 37	269 ± 35	263 ± 34	285 ± 33
Bitter	117 ± 28	127 ± 31	154 ± 33	117 ± 34
Sour	129 ± 36	110 ± 30	118 ± 32	88 ± 32
Sweet	134 ± 25	117 ± 30	103 ± 20	109 ± 24